

$\beta^2$  2. **Tyrosine phosphatases** dephosphorylate tyrosine (Tyr) residues in proteins and share a conserved active-site sequence motif Cys-X<sub>5</sub>-Arg (X = any amino acid residue) [SEQ ID NO:1] and a Asp located in a surface loop. Protein tyrosine phosphatases (PTPs) are characterized by a signature sequence motif of 11 amino acid residues, (Ile/Val)-His-Cys-X-Ala-Gly-X-Gly-Arg-(Ser/Thr)-Gly [SEQ ID NO:2] that is found in most PTPs. The diversity within the PTPs arises from the variable N- or C-terminal sequences attached to the core catalytic domain.

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Page 37, please delete the second full paragraph, and replace it with the following new paragraph:

$\beta^3$  3. **Dual-specificity phosphatases** dephosphorylate Ser/Thr residues in addition to Tyr residues in proteins. Their signature motif, His-Cys-X-X-Gly-X-X-Arg-(Ser/Thr) [SEQ ID NO:3] is analogous to PTPs but these phosphatases display a restricted substrate specificity.

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Page 59, please delete the first full paragraph, and replace it with the following new paragraph:

$\beta^4$  Total RNA was isolated from control (5.5mM glucose) or glucose-treated (25mM glucose) A10 cells and 2  $\mu$ g was used to synthesize first strand cDNA using an Oligo(dT) primer and Superscript II reverse transcriptase (Life Technologies Pre-amplification Kit). The upstream sense primer corresponded to the C4 kinase domain common to both PKC $\beta$ I and PKC $\beta$ II (5' CGTATATGCGGCCGCGTTGTGGGCCTGAAGGGG 3') [SEQ ID NO:4] and the downstream antisense primer was specific for PKC $\beta$ I (5' GCATTCTAGTCGACAAGAGTTTGTCTAGTGGGAG 3') [SEQ ID NO:5] (Chalfont *et al.*,

64 1995, pp.13326-13332.). These primers detect inclusion of the PKC $\beta$ II exon in the mature mRNA as well as PKC $\beta$ I mRNA. Sense and antisense primers for  $\beta$ -actin (#5402-3) were obtained from Clontech. PCR was performed using ampliTaq Gold DNA polymerase from Perkin Elmer (#N808-0240) on 10% of the reverse transcriptase reaction product. Following 35 cycles of amplification in a Biometra Trioblock thermocycler (PKC $\beta$ I and  $\beta$ II: 95°C, 30 sec; 64°C, 2min for 35 cycles; and for  $\beta$ -actin: 94°C, 1 min; 58°C, 1 min; and 72°C, 3 min for 35 cycles), 25% of the PCR reaction was resolved on a 1.2% agarose gel. Bands were observed under UV light and photographed.

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✓  
**Page 60, please delete the first full paragraph, and replace it with the following new paragraph:**

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65 For the stability reporter system,  $\beta$ -globin primers were designed. The sense primer was (5' GCATCTGTCCAGTGAGGAGAA 3') [SEQ ID NO:6] while the antisense primer for  $\beta$ -globin was (5' AACCAGCACGTTGCCCAGGAG 3') [SEQ ID NO:7]. PCR was performed using ampliTaq Gold DNA polymerase from Perkin Elmer (#N808-0240) on 10% of the reverse transcriptase reaction product. Following 25 cycles of amplification in a Biometra Trioblock thermocycler (94°C, 1 min; 58°C, 1 min; and 72°C, 3 min for 25 cycles), 25% of the PCR reaction was resolved on a 1.2% agarose gel. Bands were observed under UV light and photographed. The expected size of the amplified product was 320 bp.

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**Page 62, please delete the first full paragraph, and replace it with the following new paragraph:**

The 404bp PKC $\beta$ II product was obtained by PCR amplification using sense primer to the upstream PKC $\beta$  common C4 domain (5' CGTATATGCGGCCGCGTTGTGGGCCTGAAGGGG 3') [SEQ ID NO:8] and anti-sense primer to  $\beta$ IV5 domain (5' GCATTCTAGTCGACAAGAGTTTGTTCAGTGGGAG 3') [SEQ ID NO:9] such that the exon-included PKC $\beta$ II mRNA was amplified. This PKC $\beta$ II cDNA piece was cloned into the pCR-Blunt vector (Invitrogen) such that the sense transcripts could be generated from the upstream T7 RNA polymerase promoter. A 410 bp  $\beta$ -globin segment cloned into the pCR-Blunt vector was used as a non-specific competitor probe.

Page 84, please delete the fourth full paragraph, and replace it with the following new paragraph:

5'TTTTAAACCAAAGCTTTTTGGGCGAAACGCTGAACTTCGACCGGTTTTTCACCC  
GCCATCCACCAGTCCTAACACCTCCGACCAGGAAGTCATCAGGAATATTGACCAATC  
AGAATTCTGAAGGATTTCTTTGTAACTCTGAATTTTAAAACCCGAAGTCAAGAGC  
TAGTAGATCTGTAGACCTCCGTCCTTCATTTCTGTCATTCAAGCTCACAGCTATCATG  
AGAGACAAGCGAGACACCTCTCCCACTGACAACTCTGTCGACTAGAATGCCCTGA  
ATTCTGCAGATATCCATCACACTGCG 3'

Page 84, please delete the fifth full paragraph, and replace it with the following new paragraph:

Figure 27. PKC  $\beta$ II cDNA (350 bp) sequence [SEQ ID NO:10]

Page 125, please delete the second full paragraph, and replace it with the following new paragraph:

$\beta^9$  UUUUAAACCA AAAGCUUUUU GGGCGAAACG CUGAAACUUC GACCGGUUUU  
UCACCCGCCA UCCACCAGUC CUAACACCUC CGACCAGGAA GUCAUCAGGA  
AUAUUGACCA AUCAGAAUUC GAAGGAUUUC CUUUGUUAAC UCUGAAUUUU  
UAAAACCCGA AGUCAAGAGC UAGUAGAUCU GUAGACCUCC GUCCUUCAU  
UCUGUCAUUC AAGCUCACAG CUAUCAUGAG AGACAAGCGA GACACCUCCA  
ACUUCGACAA AAGUUCACCA GGCAGCCUGU GGAACUGACU CCCACUGACA

$NE$  Page 126, please delete the first full paragraph, and replace it with the following new paragraph:

$\beta^{10}$  **Figure 43.** PKC $\beta$ II mRNA sequence [SEQ ID NO:13] linearized at 175 bp with *Bgl II* and RNA secondary structure analysis.

$NE$  Page 126, please delete the second full paragraph, and replace it with the following new paragraph:

$\beta^{11}$  UUUUAAACCA AAAGCUUUUU GGGCGAAACG CUGAAACUUC GACCGGUUUU  
UCACCCGCCA UCCACCAGUC CUAACACCUC CGACCAGGAA GUCAUCAGGA  
AUAUUGACCA AUCAGAAUUC GAAGGAUUUC CUUUGUUAAC UCUGAAUUUU  
UAAAACCCGA AGUCAAGAGC UAGUA

$NE$  Page 127, please delete the first full paragraph, and replace it with the following new paragraph:

$\beta^{12}$  **Figure 44.** PKC $\beta$ II mRNA sequence [SEQ ID NO:14] linearized at 137 bp with *Hpa I* and RNA secondary structure analysis.

$NE$  Page 127, please delete the first full paragraph, and replace it with the following new paragraph: